MRID No. 429024-02

DATA EVALUATION RECORD

- 1. CHEMICAL: Oxine Copper. Shaughnessey No. 024002.
- 2. TEST MATERIAL: 1) Quinolate 98; oxine copper or copper 8quinolinolate; Batch No. 52390; 100% active ingredient; a green powder. 2) 14C-oxine copper; Lot No. 029F9233; specific activity of 92.9 μ Ci/mg; 98.25% active ingredient.
- **STUDY TYPE:** 72-3. Estuarine Shrimp Flow-Through Acute 3. Toxicity Test. Species Tested: Mysid Shrimp (Mysidopsis bahia).
- CITATION: Ward, G.S. 1993. Oxine Copper (Copper 8-Quinolinolate): Acute Toxicity to the Mysid, Mysidopsis bahia, Under Flow-Through Test Conditions. Laboratory Project ID. J9006014j. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by LA QUINOLEINE et ses dérivés, S.A., Paris, France. EPA MRID No. 429024-02.
- REVIEWED BY:

Mark A. Mossler, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.

6. APPROVED BY:

> Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.

Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA

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CONCLUSIONS: This study is not scientifically sound and 7. does not meet the guideline requirements for a flow-through estuarine shrimp toxicity study. Differences in DO concentrations between control and treatment solutions and uncertainty in actual concentrations negate the validity of the study. Under the conditions of the test, the 96-hour LC_{50} of 49.7 μ g ai/l (based on mean measured concentrations), classifies oxine copper as very highly toxic to mysid shrimp. The NOEC was 6.8 μ g ai/1.

- 8. <u>RECOMMENDATIONS</u>: N/A.
- 9. BACKGROUND:
- 10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
- 11. MATERIALS AND METHODS:
 - A. <u>Test Animals</u>: Post-larval mysids (Mysidopsis bahia), <24 hours old, were obtained from in-house cultures. The adults were maintained at a temperature of 25 ±2°C and a salinity of 20 parts per thousand (ppt). The mysids were fed live brine shrimp nauplii. Mysids appeared in good condition at test initiation.
 - B. Test System: The test was conducted using a proportional diluter system with a dilution factor of approximately 60%. The test vessels were 24-1 glass tanks which held 15 l of test solution for a resultant depth of 13 cm. The mysids were held in screened retention chambers within each test vessel.

The diluter delivered 1.4 l of test solution to each vessel per cycle (4.2 cycles per hour) for a total of 9.4 volume additions per day. The test vessels were randomly positioned in a water bath under a 16-hour light/8-hour dark photoperiod with 15-minute dawn and dusk simulations. Light intensity during the test was 325-442 lux.

Natural filtered seawater adjusted to a salinity of 20 ppt was used as test dilution water.

A stock solution containing 0.7% radiolabeled material and 99.3% unlabeled material was prepared in acidified dimethylformamide (DMF). The concentration of oxine copper in the stock solution was 1 mg active ingredient (ai)/ml. The stock solution was pumped into the diluter mixing chamber providing a high nominal test concentration of 50 μ g ai/l. The mixing chamber solution was proportionally diluted to provide the lower-concentration treatment solutions.

C. <u>Dosage</u>: Ninety-six-hour, flow-through test. Based on results of preliminary testing, five nominal concentrations (6.62, 11.0, 18.4, 30.7, and 51.1 μ g ai/1), a dilution water, and a solvent (50 μ l DMF/1) control were selected for testing.

Design: Mysids were impartially added, by twos, to each screen chamber, until 20 mysids were distributed to each treatment or control chamber. The mysids were fed live brine shrimp nauplii daily. Test solutions were not aerated during the study.

Observations of mortality and sublethal responses were made every 24 hours. Dead mysids were removed at each observation period. The temperature of the water bath was monitored continuously with a minimum/maximum thermometer and the temperature of a control vessel was measured hourly with a recording thermometer. The dissolved oxygen concentration (DO) and pH of the test solutions were measured daily in each chamber that contained live mysids. Salinity was measured daily in the dilution water control.

Samples were collected one day after test initiation and at termination for analysis of the test material by liquid scintillation counting.

- **Statistics:** The median lethal concentration (LC₅₀) and associated 95% confidence interval (C.I.) were calculated using a computer program that employed multiple analysis procedures (i.e., probit and logit analysis, moving average angle, non-linear interpolation).
- 12. REPORTED RESULTS: The mean measured concentrations were 6.82, 10.4, 17.6, 30.4, and 49.7 μ g ai/l (Table 1, attached). These values were 95-103% of nominal concentrations.

The responses of mysid shrimp are given in Table 2 (attached). The 96-hour LC₅₀ based on mean measured concentrations was 49.7 μg ai/l (95% C.I. = 30.4 μg ai/l to infinity). The no-observed-effect concentration (NOEC) was reported to be 10.4 μg ai/l.

Dissolved oxygen ranged from 3.2 to 7.3 mg/l or 40 to 92% of saturation in the treatment and control solutions. The pH values ranged from 7.9 to 8.5. The temperature ranged from 20.6 to 21.6°C.

13. <u>STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:</u>
The author presented no conclusions.

Good Laboratory Practice (GLP) and Quality Assurance statements were included in the report indicating compliance with EPA GLP Standards, 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. <u>Test Procedure</u>: The test procedures deviated from the SEP as follows:

The recommended temperature for mysid shrimp toxicity tests is 22 $\pm 1\,^{\circ}$ C. The temperature in the study was 20.6-21.6°C.

The salinity of the dilution water in the study was 20 ppt with a pH of 7.9-8.5. The recommended salinity and pH for estuarine shrimp tests are 10-17 ppt and 7.7-8.0, respectively.

The DO in two or more treatment solutions dropped below 60% of saturation for the last three days of the study. The SEP states that the DO in flow-through tests must remain between 60 and 100% of saturation.

The results of the salinity measurements were not reported.

- B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained similar results (see attached printout).
- C. <u>Discussion/Results</u>: There was a large difference between the DO concentrations in the control and treatment solutions (Table 5, attached). Additionally, the initial chemical concentrations (within the first 24 hours) were unknown due to the lack of samples collected at test initiation (page 15, attached). These variations and uncertainties question the validity of the obtained LC and NOEC values.

This study is not scientifically sound and does not meet the guideline requirements for a flow-through estuarine shrimp toxicity study. Under the conditions of the test, the 96-hour LC₅₀ of 49.7 μ g ai/l (based on mean measured concentrations), classifies oxine copper as very highly toxic to mysid shrimp. Since signs of toxicity were noted at the 10.4 μ g ai/l treatment level, the NOEC would be reported as 6.8 μ g ai/l.

D. Adequacy of the Study:

- (1) Classification: Invalid.
- (2) Rationale: Differences in DO concentrations between control and treatment solutions and

uncertainty in actual concentrations negate the validity of the study.

- (3) Repairability: No.
- 15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 11-17-93.

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MOSSLER OXINE COPPER MYSIDOPSIS BAHIA 11-17-93 ************************ NUMBER PERCENT BINOMIAL EXPOSED DEAD DEAD PROB. (PERCENT) 49.7 20 10 50 30.4 58.80985 20 0 0 17.6 9.536742E-05 20 1 5 10.4 2.002716E-03 20 0 0 9.536742E-05 6.8 20 0 9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 49.7

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

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6 .3956043 1 .1025742

SLOPE = 4.737182 95 PERCENT CONFIDENCE LIMITS = 1.757633 AND 7.716732

LC50 = 54.3039 95 PERCENT CONFIDENCE LIMITS = 43.66915 AND 104.7473